INDEXED. PATH. COLL.

The Angular Leaf Spot of Cotton

R. C. FAULWETTER,

Associate Botanist and Plant Pathologist

BULLETIN 198

SOUTH CAROLINA

AGRICULTURAL EXPERIMENT STATION

OF

CLEMSON AGRICULTURAL COLLEGE

H. W. BARRE, Director

CLEMSON COLLEGE, S. C. FEBRUARY, 1919.

CLEMSON AGRICULTURAL COLLEGE
W. M. RIGGS, President

SOUTH CAROLINA EXPERIMENT STATION

BOARD OF CONTROL

HON. B. R. TILLMAN,
HON. J. E. WANNAMAKER,
HON. B. H. RAWL,
HON. ALAN JOHNSTONE,
HON. R. I. MANNING,
HON. A. F. LEVER.

EXPERIMENT STATION STAFF

H. W. BARRE, A. M., Botanist and Director.
C. C. NEWMAN, B. S., Horticulturist.
A. F. CONRADI, M. S., Entomologist.
G. F. LIPSCOMB, Ph. D., Chemist.
----------, Animal Husbandman.
R. O. FEELEY, D. V. S., Consulting Veterinarian.
W. W. FITZPATRICK, B. S., Dairyman.
C. P. BLACKWELL, M. S., Agronomist.
A. B. BRYAN, B. S., B. Litt., Agricultural Editor.
F. C. HARE, Poultry Husbandman.
R. C. FAULWETTER, B. A., Associate Botanist and Plant Pathologist.
W. B. AULL, B. S., Associate Bacteriologist.
W. J. YOUNG, M. S., Associate Horticulturist.
E. E. HALL, M. S., Expert in Cotton Breeding.
J. L. SEAL, M. S., Extension Pathologist.
G. H. COLLINGS, M. S., Assistant Agronomist.
C. L. MORGAN, B. S., Assistant Animal Husbandman
W. R. HOOTS, B. S., Research Assistant in Horticulture.
MISS ELLEN CONVERSE, M. S., Assistant in Botany.
M. R. SMITH. M. S., Assistant Entomologist
J. O. GRAHAM, B. S., Assistant in Chemistry.
J. A. RILEY, M. S., Supt. of Coast Experiment Station. Summerville, S. C.
R. E. CURRIN. Supt. of Pee Dee Expt. Station, Florence, S. C.
BURNS GILLISON, Foreman of Experiment Station Farm.

Mail and Telegraph Offices: Clemson College, S. C.
Freight and Express Offices: Calhoun, S. C.

The Bulletins and Circulars of the Station are issued at irregular intervals and are sent free to all citizens of the State who apply for them.

The Angular Leaf Spot of Cotton

INTRODUCTION.

The history of the investigation of this disease is short, and a brief outline of the main points must suffice as an introduction to the experimental work which has been done by the author and which has led to the determination of a successful control. Atkinson (1) mentioned the occurrence of "definite areolate spots which are bounded by the veinlets of the leaf. The spots are sometimes very numerous and frequently conjoined; often the disease follows one or more of the main ribs of the leaf, being bounded on each side by an irregularly zigzag line." Later (2) he used the name "Angular Leaf Spot" which is particularly descriptive of the most conspicuous phase of the disease; i. e., on the foliage leaves. Smith (12) mentioned a yellow flagellate organism which he had demonstrated to be the cause of this disease and designated it as *Bacterium malvacearum*. This brief note hardly deserves the name of a decsription for, excepting its statement of the pathogenicity of the organism on cotton leaves and a few morphological data, nothing is included that would permit identification or comparison. Edgerton (4) included a discussion of this disease with considerable experimental data on other boll diseases of cotton. Rolfs (12) presented the early results of this investigation which since was assigned to the writer.

THE DISEASE.

The disease is most conspicuous upon the foliage leaves, but occurs upon the cotyledons, stalks, petioles, bracts, and bolls. In each of these instances the outline and appearance of the lesion differs slightly, but the characteristic watery, dark green, viscid spots can not be mistaken in any case. The first evidence of infection upon the leaves appears as minute, dark green, angular (triangular or quadrilateral) spots on the under side. A day or two later the spots appear upon the upper side, though less conspicuously angular. It usually increases in size simultaneously on both sides of the leaf but never crosses the larger veins. Invasion of neighboring areolate spots bounded by the smaller veinlets accounts for the

enlargement of the spots shortly after their appearance. Single infections seldom become more than 3 to 4 millimeters across. Plate I. The zigzag spots mentioned by Atkinson are undoubtedly caused by a coalescence of numerous separate infections made more easily possible by the persistence of water at such places during rains and dews. Plate II.

The upper side of the leaf spot soon becomes reddish brown in color and shrinks slightly in thickness. The underside turns brown and is bordered by a narrow band of dark watery green. Finally the color changes over the whole lesion on both sides and the infected area dries out, retaining a hardened, scaly surface.

The lesions upon the cotyledons are not angular in outline. This is probably due to the fact that the cotyledons are different in structure, being thick, leathery, smooth on both sides, and lacking the finely net-veined skeleton of the foliage leaf. The spots here are curcular or oval, with an indefinite margin, green at first but becoming black later without the color changes which occur on the foliage leaves.

The lesions on the bolls resemble very closely those upon the cotyledons, being dark green at first with an indefinite margin, but soon becoming black, sunken, wrinkled, and having a very definite demarkation from the remainder of the boll surface. They may attain a diameter of 5 to 10 millimeters.

On the bracts, the lesions are larger than in any other place but occupy the space between the course veinlets, which are almost rectangular. Single lesions may become two centimeters long and half as wide, the infected tissue becoming brown and papery.

Few instances of the disease upon the stalks and petioles have been observed by the writer, but Orton (11) indicates that this stage is common upon Sea Island Cotton in some localities. In the instances observed, the lesions have been large with an irregular outline, extending three or four centimeters along the stalk and half way round. Here the disease most appropriately bears Smith's (13) comparison to the disease of bean pods caused by *Bacterium phaseoli.*

THE ORGANISM.

The organism causing this disease *Bacterium malvacearum,* Smith (13), was perhaps sufficiently designated by its ability to cause the "characteristic leaf disease (of cotton) known

as Atkinson's Angular Leaf Spot", and this ability is in the last analysis the most necessary and distinctive criterion for identification. Subsequent writers have postponed a more complete description, but the writer saw the need of recording notes upon the subject. Smith says, (13) that it morphologically resembles the other yellow, one flagellate rods causing diseases of walnut, bean, hyacinth, citrus, etc. It is in the form of short rods, usually occurring in pairs or chains of three and sometimes more. The flagellae have been observed in young cultures. One, sometimes branched at the end, or occasionally two flagellae occur at one end, making possible very active motility. No endospores, granules, or capsules have been demonstrated. The so-called cultural characters have been described by the writer (5), but a brief summary of these notes is included here for completeness.

Moderate growth takes place on all the sugar agar slant media. In each, the surface of the streak is slightly convex, smooth, homogeneous, and glistening, the extent of the growth, its marginal outline and the shade of color depending on the sugar need. On plain agar the color is very pale and the outline irregular. Of the sugar agar slants the most growth takes place on saccharose, being followed closely in amount by that on dextrose, in both of which the streak spreads almost to the edge of the medium throughout its entire length and has the most entire margin, while the water of condensation is completely filled. Next in order of development come dextrin, maltose, lactose, manite, and plain agar. In these there is more pronounced spreading at the base of the slants, extending to the edge of the medium in each case, though the water of condensation is not filled. In the dextrin and lactose cultures the films and precipitates are thick and almost meet, while on mannite the growth occupies less space and on maltose and plain agar cultures the film is very thin and the precipitates are slight. The pale yellow color varies in each, the deepest yellow being found in dextrose, and the others lighter in the following order: lactose, saccharose, dextrin, maltose, mannite, and plain agar. In none was any odor developed differing from that stale odor characteristic of most bacterial cultures.

In liquid cultures growth was most abundant in the case of 1 per cent saccharose broth and next in dextrose broth. This confirms the preference for those sugars noted in the cultures on agar slants. Little difference was to be noted among the rest of the media used except that growth was poor-

est in sugar-free broth and glycerine. The latter was made of sugar-free broth and contained no carbohydrates. In none was any characteristic odor, or sign of gas developed. Acid was not formed, but the original acidity was reduced.

Tests of sugar-free broth cultures of this organism fifteen days old, for indol with the nitrosal-indol reaction, and for phenol with ferric chloride, Millon's regeant and bromine water separately were all negative.

All broth media were originally corrected to 1 per cent normal acidity, but after fifteen days' growth, the titre was lowered, varying from neutrality to 1 per cent. Acids were not formed but amomnia was found upon testing with Nessler's reagent in all cases. The amount of ammonia was roughly dependent upon the media used. Gas was not formed in any instances. Gelatine was slowly and but slightly liquefied. Slow growth also took place in litmus milk, the separation of clot and whey taking place without curdling. The clot was finally pale lavendder, occupied the power part of the tube, was firm and friable, and easily cut with a platinum needle. Diastatic action on starch in potato slugs and starch jelly was strong. Potassium nitrate, litmus, and neutral red were reduced, the first most quickly. The organism will tolerate no more than 2 per cent normal acid broth. It grows on 2 per cent sodium chloride broth but no greater concentration. One percent phenol, 1 per cent formaldehyde, .01 per cent mercuric chloride, or 80 degree C. will each kill the organism in ten minutes.

A statement in regard to the use of the generic term *Bacterium* in this connection is not out of place here. Were all bacteriologists to use Migula's system in its entirety, the term *Pseudomonas* would be accepted, but since many modify Migula's system as they see fit, well founded exceptions will bear consideration when an agreeable code is adopted. Hence Smith's (14) claim of the priority of Cohn for the use of the term *Bacterium* is well founded, and, if priority will have any weight with bacteriologists as a whole, undoubtedly this term will be accepted.

THE SEASONAL HISTORY.

One can not use the term life history or life cycle in describing a disease of this sort, because first, a disease, as such, has no life, and second, the organism causing the angular leaf spot of cotton has but one stage which constantly reproduces itself by direct fission so far as is known at present. It would

be interesting to repeat the experiments of Lohnis and Smith (10) using a plant parasite which resists dessication in winter, as this one does upon the lint, while it can not resist shorter dessication in summer or live as long a time in culture media. Possibly there is another phase of the life cycle in this case but it remains to be demonstrated. Rolfs (12) says "the bacteria appears to remain dormant as long as the seed is in the resting state. However, as soon as the seed is planted and conditions become favorable for germination, the bacteria also become active and soon attack the growing tissues—. The seed in infected bolls usually mature and when planted, germinate and grow. Such seed may harbor the organism in the inner tissues and when planted transmit the disease to the young seedling." There is no doubt that the organism lives over the winter, but no evidence is at hand or presented in the literature to support the conclusion that internal seed infection occurs.

The seasonal history is divided into two phases—(1) the primary infection upon the cotyledons and (2) the secondary infections upon the foliage leaves, stems, bracts, and bolls. As will be discussed later under the subject of control, it has been demonstrated that the primary infections can be prevented and in such cases with no infected cotton near by, the disease can be controlled.

Under the usual field conditions, one or both cotyledons on a low percentage of the seedlings have bacterial lesions. This number differs, of course, with each lot of seed and depends upon the severity of the disease during the previous season. The term "primary lesion" can be strictly applied only to those spots resulting from inoculation by bacteria which came directly from the lint of the seed which had survived the winter. It is difficult to estimate accurately the influence of environmental conditions upon the success of inoculation by these bacteria. Warm rainy weather would seem to favor and cold dry weather lessen such infections. Those spots appearing within 15 to 20 days following germination have been considered as primary, and all later spots as secondary, but there is no way of proving which is one or the other. It is obvious that a few secondary infections may occur in this time (seven days being the minimum reported or observed for the appearance of the lesions after artificial inoculation) and also that some primary infections, being slow in developing, may appear later. Within this period, the percentage of infected plants has always been extremely low.

In the following table, a series of records of the infected

seedlings in various lots of seeds shows this point clearly and also the great increase in the immediately following period.

TABLE I.

Showing slight amount of cotyledonary infection and rapid increase shortly afterward.

No. Plants	Date Planted	Percentage Infected		
		10 days	16 days	28 days
656	May 24	0.45	2.68	38.3
1119	May 24	0.0	4.28	50.7

Those factors which seemed most probably concerned in the hibernation of the organism and the appearance of the primary lesions are:

(1) Presence of the bacteria in the soil.
(2) Survival in decaying cotton plants and plant parts.
(3) Contamination of short lint or fuzz upon the seed.
(4) Internal seed infection.
(5) Hibernation upon or within insects.
(6) Susceptibility of plants closely related to the cotton plant.

All of these factors are intermingled in the subsequent experiments, but they will be discussed separately as far as is possible.

The longevity of the organism in the soil under field conditions was investigated from several angles. A wooden box filled with sand, six inches deep and thirty inches square, was placed in the ground with its top level with the surface. Several liters of a thick suspension of bacteria in water were poured over it about the 25th of August, 1916. Poured plate isolations from this soil during the following December and at frequent intervals afterwards failed to develop colonies of the organism. In the following spring seeds known to be free of the disease were planted in this sand and kept well watered for a month following germination. The disease did not appear during that time.

The possibility presented itself that the winter conditions in the Piedmont section of the State might affect more severely the ability of the organism to survive than the milder weather of the eastern section. Seed of the lot known to be free of the disease were planted in a field in a southeastern county, in which badly diseased cotton had been grown the previous summer. But of 326 seedlings, six weeks old, which had been subjected to several showers that could have beaten

the cotyledons down to the ground or splashed surface water upon the leaves, none were diseased. This experiment is not conclusive, but when considered with the results obtained at the Experiment Station, it supports the opinion that the organism does not live long in the soil after being washed from active lesions to the ground or liberated thru the decay of diseased leaves.

Opportunity was given to determine the ability of these bacteria to subsist thru the winter in the decaying plants and plant parts. In the fall of 1916, some 25,000 bolls upon several thousand plants were inoculated to afford a supply of diseased lint and seed for further study. In due time these bolls ripened and the lint was picked. From time to time during the winter a number of the empty capsules were collected and taken to the laboraotry. Isolations were attempted from the plainly discernible diseased area surrounding the needle punctures, but none were successful. In several instances such capsules were gathered from the ground where they had fallen, under the supposition that the moisture of the soil might have some influence upon the length of life; but colonies of the organism did not appear upon any of the plates.

Internal seed infection was studied after the method described by Barre and Aull for determining cotton seed infections by anthracnose, caused by *Colletotrichum gossypium* South. Test tubes containing bits of saturated blotting paper to the depth of an inch or ann inch and a half were sterilized in the autoclave. Seed from the inoculated bolls mentioned above were delinted with sulphuric acid and the smooth seed coats washed thoroughly with soap, rinsed in water and treated with mercuric chloride solution (1 part in 1000 parts of water) for ten minutes. After repeated rinsing in water they were planted singly in the test tubes. Among more than one thousand seedlings grown in this way several suspicious spots occurred upon a few of them, and realizing that the appearance of disease might be more or less atypical when developed under such conditions, isolations were made from each of these, but *Bacterium malvacearum* was not found in any instance. The experiments later, under the discussion of lint contamination, also confirm the conclusion that internal infection of the seed occurs if at all in very rare cases.

In order to secure a lot of infected seed for these experiments, extensive inoculations of bolls were made during the fall of 1916. The lint from these bolls was gathered in two

lots; (a) that from the punched or inoculated locules, and (b) that from the uninoculated locules of these bolls. Seed development in the inoculated locules was interrupted in many cases and consequently percentage germination was lowered, but of these seedlings appearing from both lots after planting, little difference in the amount of infection was noted. Much of this may be due to the gin, both lots having been ginned at the same place and time, but without doubt the bacteria spread from the point of inoculation over all the lint of such bolls.

Primary infection by bacteria carried over the winter by insects probably does not occur in this disease. Besides the experiments described above, which were conducted with such a limited number of plants, an acre plot in a pasture field, which had never been previously used for cotton, was planted with seed of the lot mentioned above as being free from disease. This plot was arranged for use in the writer's studies upon the dissemination of the disease, and after two careful examinations of the seedlings before "chopping", no case of the disease was found. The insect fauna of this field was doubtless typical of other cotton fields upon the farm, and sufficient opportunity was offered for infection by this means if such occurs.

The question of hosts other than the cotton plant has not yet been investigated fully. Very few of the wild Malvas occur in this vicinity. Of these *Sida spinosa* is the most common. Cultivated forms of some *Hibiscus* species are frequently grown, though nothing resembling the angular leaf spot of cotton has been found or reported upon them. It seems highly improbable that any of these plants are susceptible to this disease in view of the work upon the subject by Rolfs, Edgerton, Smith and others, though it is not mentioned in any of the publications of these men that this particular phase of the problem has been investigated

The statement of Smith (14) that the younger tissues are more susceptible to infection than the older part has been confirmed. In the inoculation studies made, it was shown that the underside of the young growing leaves was the most susceptible part of the entire plant. A few lesions appear after seven or eight days' incubation and the number increases constantly through the fourteenth day, after which it is impossible to determine whether the latter spots are the results of the original inoculation and have had an unusually long incubation period or are new infections by bacteria from the first spots.

Two series of inoculations were made to demonstrate this point, the first during the unusually rainy July of 1916 in which it rained every day from the fifth to the twenty-fifth, and the second at the end of that period, the disease developing during the drier weather. The results of this work indicate the comparative susceptibility of the parts inoculated and the relative influence of the prevailing meteorological conditions. The data are arranged in the following tables.

TABLE II.

The average number of angular leaf spots per leaf on the eighth to sixteenth day inclusive, following inoculation. Very wet weather. (100 leaves used in each instance.)

	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days
Large leaves									
top side	1.71	2	6	9.4	9.9	12.9	15.3	22	29
bottom side	.93	2.2	6.2	9.	9.3	12.4	13.3	17.3	26
Small leaves									
Top side	1.4	3.3	4.3	6	9.9	10	11.3	12.8	20
bottom side	5.6	9	19.7	34.8	42.8	57	58	58	82

TABLE III.

The average number of angular spots upon each of fifty or more leaves of various ages.

	10 days	12 days	14 days	16 days	17 days
Leaves one day old	.32	2.17	3.6	7.5	9.3
Leaves four days old	0	.08	1.06	4.58	5.02
Leaves seven days old	0	0	0	.31	.72
Leaves nine days old	.03	.03	.1	.3	.4

TABLE IV.

The average number of spots upon each of approximately 100 large leaves inoculated during dry weather and wet weather.

	9 days	11 days	14 days	15 days
Wet weather				
top side	2	9.4	15.3	22
bottom side	2.2	9.9	13.3	17.3
Dry weather				
top side	2.3	2.2	2.4	2.9
bottom side	.86	2.9	6.5	8.7

By examination of table II it will be noted that inoculations with the same suspension of bacteria and at the same time, upon the under side of small leaves—one to two days

old resulted in more than four times as many spots upon any given day (excepting the ninth) than upon the top sides. The latter compared favorably with the results of inoculations of large leaves—either top or bottom side.

Table III contains similar data showing the greater susceptibility of the younger leaves, all being inoculated at the same time and with the same inoculum. Leaves were tagged when one day old, and when those tagged first had reached the ninth day all were inoculated. It will be noted also that the incubation period seems to be influenced by the age or susceptibility of the leaves.

An explanation of the difference of susceptibility has been sought in some of the possible differences in the smaller and larger leaves, but as yet no one condition has been determined to exert an influence in this direction.

Smith (14) presents evidence that infection takes place thru the stomata, and the possibility that the stomatal number per unit area might be relatively greater in the case of smaller leaves than larger leaves and thereby account for the increased susceptibility led to the determinations of this data. It was found that stomotal formation begins shortly after the very small leaf is unfolded and continues rapidly for several days, making the largest number per unit area about the sixth to tenth day and the total per leaf reaching the maximum about the tenth to twelfth day (Tables V and VI). Inoculations in the experiments mentioned above were made upon leaves three to four days old which did not have as many stomata per unit areas as the larger leaves.

TABLE V.

Area, stomata per unit area and per leaf. Average of five determinations on each side of ten leaves of each age. All leaves one day old on June 26, 1917.

Age of leaf	Area of leaf in sq. cm.	Average no. stomata per sq. mm. top surface	Average no. stomata per sq. mm. bottom surface
2 days	3.75	68.1	124.2
4 days	11.39	98.6	187.
6 days	26.67	150.8	273.9
8 days	42.79	146.3	304.
10 days	44.67	122.5	245.2
12 days	72.5	131.3	240.7
14 days	57.97	120.6	217.3
16 days	70.13	114.9	218.7

TABLE VI.

Leaf area, stomata per unit area and per leaf. Average of five determinations on each side of ten leaves of age. All leaves one day old on July 5, 1917.

Age of leaf	Area of leaf in sq. cm.	Averabe no. stomata per sq. mm. top surface	Average no. stomata per sq. mm. bottom surface
2 days	4.5	.5	6.4
4 days	13.8	19.6	71.
6 days	27.4	123.8	218.1
8 days	49.	154.8	278.1
10 days	93.3	154.	323.5
12 days	127.3	134.1	288.6
14 days	141.5	114.6	279.9

Since the stomatal number has been found to be less in young leaves than in old, thus eliminating large stomatal number as an important factor for successful inoculation, another cause, that of sap acidity, was investigated.

Previous studies (5) of the physiology of the organism *Bacterium malvacearum* Smith, have shown the acidity of culture media upon which the organism can grow to be equal to 2 per cent normal acid.

Determination of the acidity of the cell contents of very young leaves, and very old leaves were made during the latter part of August. A supply of each of the various-aged leaves was taken from the field, ground in a food chopper and the tissue juices expressed. The resultant liquid was diluted to ten times its original value, filtered with Kusselguhr thru filter paper and titrated against N-20 solution of sodium hydroxide using a .5 per cent solution of Phenolphthaloin as an indicator. Numerous preliminary determinations were made to perfect the method. The results, which are averages of a number of determinations, follow:

Old leaves ---------- 4.10 per cent normal acid
Middle aged leaves ---------- 5.15 per cent normal acid
Young leaves ---------- 7.80 per cent normal acid

The acid content of the younger leaves is greater than that of the older, and is furthermore much greater than that which the organism can tolerate in artificial media. Obviously, acidity alone is not the determining factor for the greater susceptibility of the younger leaves.

While studying the physiology of *Bacterium malvaccarum* and its relation to stomatal infection, the effect of the dis-

ease upon stomatal development, movement and transpiration was considered. Very young leaves were inoculated with the organism, and after infection had taken place, the epidermis was stripped from the spots, examined with a microscope and the number of stomata per square millimeter calculated. The average number per millimeter upon leaves that were equal in size to healthy leaves, was found to be lower, indicating that the disease prevents normal development of stomata when infection of very young leaves occurs.

The stomatal openings upon healthy leaves were measured at various times during the day but the only observations upon diseased areas were made between ten and eleven A. M. It is at this time, however, that greatest opening occurs in the cotton plant, as has been previously noted by Lloyd (9). The records of these observations are given in Tables VII and VIII. It will be noted that the averages and deviations are calculated from twenty-five observations in each instance.

TABLE VII.

Stomatal opening at various times of the day.

Observations	6 to 7 A. M.		10 to 11 A. M.		11 to 12 A. M.		2 P. M.	
	Dev'n	Aver.	Deviation	Aver.	Deviation	Aver.	Deviation	Aver.
25	1—2.4	2	2—3.5	3.02	1.5—3.2	2.81	0—3.2	1.36

TABLE VIII.

Stomatal opening in healthy and diseased areas.

Observation	Healthy		Diseased	
	Deviation	Average	Deviation	Average
25	2—3.5	3.02	0—3	.92

It is shown in Table VIII that the stomata upon diseased areas are practically closed at that time of day when those upon healthy areas are open widest. Stomatal transpiration appeared to be greatly inhibited by this condition, but the possibility of uncontrolled surface evaporation thru the dead tissues was realized and determinations of water loss thru such spots were made. The colbalt chloride paper method was used, and in the first preliminary tests it was found that the water given off thru the bacterial spots was much less than that thru surrounding tissues. Since the paper eventually became entirely red, elimination of diffusion of water thru the paper from the healthy tissue was necessitated. After several failures the following method was resorted to with good results. A very thin piece of rice paper was taken to

the field and placed upon some diseased leaves. The outlines of certain designated spots were traced upon the paper. These were retraced upon hard glazed paper squares and the enclosed area cut out with a sharp razor. One of these was placed upon a leaf with the hole exactly over the spot after which it was copied. Over this was placed a piece of colbalt blue, 3 per cent colbalt chloride paper and both clamped in position between glass strips. The edges of these strips were coated with vaseline to prevent entrance of atmospheric moisture to the margin of the cobalt chloride paper which otherwise turned faintly pink. In this way exposure was made only to the surface of the diseased spot and a change of color was never noted. The waxy coating over the spots caused by the bacterial exudate which is always more or less present seems to be a seal against water loss thru these spots.

INJURY.

It is very difficult to estimate the damage done by this disease. Under the conditions prevailing in the Piedmont section of the south-eastern States little boll infection occurs, never more than one percent having been observed by the writer. In the more southerly portions as many as 60 percent of the bolls have been found to be affected by this disease. Even under these conditions a true rot or decay of the lint very seldom occurs and that only under the most favorable conditions. The spots on the bolls serve as points of entrance for a number of organisms, both bacteria and fungi. The fungus spores developed over the surface of the bacterial spots on seventy-five bolls collected from a cotton patch at the Mississippi Experiment Station in September, 1917, were examined. Eighty percent of such spots had been invaded by secondary fungi.

No effort was made to determine the identity of these but they fell roughly into the following genera: Colletotrichum, Fusarium, Alternaria, Phyllosticta, and Cephalothecium. The Alternaria species, described in another place by the writer (8), was very common upon such spots. Edgerton (4) says, "The disease may be confined entirely to the tissue of the carpels or it may extend into the developing lint and seed underneath." The writer found that spots which had not been invaded by fungi seldom extended deeper than the tissues of the carpel, but in the others the lint was changed to a slight yellow-green color or completely destroyed.

The lesions upon the leaf often become so numerous as to involve almost the entire blade. Some often wither and fall off from no other obvious cause and without infection of the petiole by this or other diseases. The oldest lower leaves turn yellow when affected with but a few spots. It is this stage which the growers of Sea Island cotton term "Yellow Leaf", though the same appearance can be caused by the Areolate leaf spot (caused by *Ramularia areola*, Atkinson) when these spots become sufficiently numerous. The loss of entire leaves is an important item, but when all the leaves of a cotton patch are considered, an extremely low percentage is affected that seriously. Data are presented in the following table which show the status of affairs in the first half of the growing season. Twenty average plants were gathered in a field in which the disease was as abundant as in most fields at that date. The total leaf area of each plant and the area affected by this disease were determined by tracing the outlines of leaves and spots upon paper, weighing, and calculating from the weight of a sample of the same paper whose area had been determined. Except the possible formation of some diffusible deleterious substances in the infested spots, there is no evidence of injury except the loss of synthesizing tissue. The following tables show the small amount of tissue invaded. In Table IX are presented the determinations of the area invaded in twenty average plants during the first part of July when the plants were in the most active stage of their development. The leaves were dark green and increasing in size and number very rapidly. In Table X are shown results of similar determinations made during the middle of August when the plants had reached the height of their foliage development and shedding of leaves had become common. At the time of the first observation the disease was as abundant as it usually becomes, and the plants selected were typical representatives of the field. The relatively slight area invaded at that time will be noted, with extreme maxima of 12.6 per cent and 5.7 per cent occurring in but a tenth of the total number of plants. The average of the field is fairly indicated by the average of the plants selected; namely 1.2 per cent. At the time of the observation recorded in Table X the foliage tissue invaded is greatly reduced, because of the loss of the older infected leaves by shedding, and the increase of the spread of the disease both of which are characteristic at that part of the season.

TABLE IX.

Leaf Area Invaded in July.

Plant No.	Total leaf area sq. cm.	Area invaded sq. cm.	Percentage diseased
1	845.6	5.6	.7
2	601.4	3.6	.6
3	524.5	1.1	.2
4	601.	11.7	1.9
5	857.6	11.9	1.4
6	728.7	1.	.1
7	872.3	14.	1.6
8	553.6	.8	.1
9	953.1	53.9	5.7
10	1555.1	7.2	.4
11	1176.6	12.2	1.0
12	1039.8	131.6	12.6
13	1164.7	.35	.1
14	676.1	.8	.1
15	1470.2	.3	.1
16	917.7	.1	.0
17	2038.2	14.7	.7
18	406.8	4.2	1.5
19	878.6	.7	.1
20	1333.	.6	.1
Av.	959.7	17.0	1.2

TABLE X.

Leaf area invaded in August.

Plant No.	Total leaf area sq. cm.	Area invaded sq. cm.	Percentage diseased
1	1968	.9	.045
2	4337	1.06	.024
3	3945	6.92	.17
4	2703	7.92	.29
5	2614	3.19	.12
Av.	3113.4	3.99	.129

It remains to determine whether the yield of seed cotton is affected by the injuries due to this disease. While no doubt the plant as an individual is injured, it is possible that other limiting factors (such as nutrition, water, aeration, etc.) determine the size of the crop. The water relation of the cotton plant as studied by Barre (3) probably more than any other one factor or combination of factors controls the annual yield of cotton.

DISSEMINATION.

There are two phases of the subject of dissemination to be considered: local and general. In some early inoculation studies, records were made of the results of the inoculation upon leaves when the plants were very young and the advance of the disease upon the newer leaves as they were formed. Plants having an average of 4 to 6 leaves were inoculated from a number of slant agar cultures by painting the bacteria upon the undersides of the leaves with a cotton swab. The inoculated leaves developed the disease after the usual incubation period. Ten days after inoculation 261 leaves of a total of 571 leaves on all the plants were diseased. Ten days later 539 leaves of a total of 743 at that time were diseased. Young leaves are developed at the tips of the branches with great rapidity during the first half of the summer, and the new leaves become infected as quickly as they are formed.

Such rapid spread over the newer leaves of the same plant is undoubtedly due to the contact of the leaves when they are wet with dew or rain and this is considered here as "local" dissemination. Rolfs (12) describes the process as follows: "Usually, by the time the second leaf has developed sufficiently to come in contact with the first true leaf, the disease will have also advanced sufficiently to inoculate the second leaf, and by the time the third leaf reaches the second, the disease on the second will have developed sufficiently to inoculate the third, and so on until most of the leaves will have been inoculated. If for any reason the first leaves fail to become inoculated in this way, the movement of the leaves in the wind, especially during a storm, will soon bring them in contact with some of the virus on the diseased leaves." This combination of conditions progressively inoculates the leaves as they are developed. Insects, birds, and laborers also have a part in this spread, yet it must be a small one since in the experiment described above but three uninoculated plants in the same row had any spots upon them, one apiece in each of two cases ,and four upon the other.

The more general dissemination of this disease has been a subject of particular interest to the writer. From the results of the experiments recorded in the following table, it was noted that a very low percentage of the seedlings have lesions upon the cotyledons in a period of time ample to permit of infections from the bacteria upon the seed coat and lint but before secondary spread from these has occurred.

TABLE XI.

Record of plantings and diseased plants at various intervals thereafter.

Exp.	No. of Seedlings	Date Planted	Date Rec.	Percent Disease	Date Exam.	Percent Disease	Date Exam.	Percent Disease
1	656	May 14	May 24	.45	May 30	2.68	June 11	38.3
2	1119	May 14	May 24	0.00	May 30	2.48	June 11	50.7
3	890	May 21	June 9	9.40				
4	154	Aug. 12	Aug. 22	0.00	Sept. 2	1.30		
5	378	Aug. 12	Aug. 22	1.00	Sept 2	1.80		
6	279	Aug. 12	Aug. 22	0.00	Sept. 2	.80		
7	329	Aug. 12	Aug. 22	1.00	Sept. 2	1.00		
8	486	Aug. 12	Aug. 22	0.00	Sept. 2	0.00		
9	336	Aug. 12	Aug. 22	0.00	Sept. 2	2.70		
10	4282	Mar. 27	Apr. 25	2.80	May 7	6.40	July 17	24.0
11	6240	May 2	June 10	28.4	July 10	98.		

The most severely infected lot of seed observed by the writer showed but 9.4 percent seedlings with spotted cotyledons. The great increase of percentage of diseased plants where subsequent records were made indicates the rapid and wide-spread manner in which disseminattion occurs. That 98 to 100 percent of the plants should be infected from as low as 10 percent in thirty days seems impossible but it is nevertheless true.

Insects were observed very closely in the beginning of the work on dissemination. As has been reported before (6), little evidence is at hand to indicate such activity. Cotton was planted near infected fields under cages to prevent insect visitation, and no less disease appeared under the cages than upon the plants outside. Jasids, the most abundant visitors to the plants while they are wet with dew, were caught and caged upon susceptible plants. But one spot appeared in a caged plant and that with 75 insects crawling over it, while a sprayed check plant under a cage had 871 spots in the same time.

It is the writer's opinion that insects play a more important part in the spread of the disease upon the bolls. The bolls are much more resisant to inoculation without wounding than the leaves. In the Piedmont of South Carolina, very little boll infection occurs. Edgerton (4) emphasizes the relation of insects to this stage of the disease. He says: "There are a number of insects that puncture the cotton bolls and aid the bacteria in gaining entrance to the boll contents. A considerable percent of the water-soaked spots produced by

Bacterium malvacearum, especially in older bolls, have these small punctures at the center." Thru correspondence with the entomologists in the various cotton states it has been found upon good authority that *Leptoglossus phyllopus* Linn., *L. oppositus* Say, and *Cotocoris rapidus* Say, occur abundantly in the central southern staates on cotton bolls and are relatively rare or do not occur at all in this section of South Carolina. On this type of deduction it seems probable that insects are a controlling factor in boll infection.

There is little need to repeat the data presented by the writer (6) (7) in two previous publications, relating to the influence of the wind during rain upon spread of the disease. It has been shown that the bacteria emanate from the lesions and can be found in the dew film, that the splash droplets liberated by a falling drop are made up primarily of the surface film ,and are scattered in enormous quantities, that a drop of water the size and falling with the velocity of a rain drop can scatter splash drops as far as 40 inches in quiet atmosphere, that the angle and elevation of the leaf surface influences the distance of splash, that a wind moving at the rate of 10 miles per hour will blow drops splashed from a surface three feet from the floor as far as 18 feet and that the disease can spread as far as 80 feet from inoculated plants when the proper conditions prevail. Undoubtedly water droplets will travel in direct proportion to the elevation of the surface from which they splash and the velocity of the wind. It remained to repeat the field observations under registered conditions and to show that the wind does blow fast enough during the average Southern thunderstorm and shower to bring about these results. It was originally planned to incorporate these data in another paper which would have completed the series begun on the subject of wind and rain dissemination of this and similar diseases, but they will be included here.

A square acre plot crowning a broad hill openly exposed on all sides was prepared for cotton planting in the spring of 1917. In half of the field the rows extended from northeast to south- west and in the other half almost at right angles to these. In the center of the plot a standard Robinson *anemometer was placed upon a post six feet high, and

*The meterological instruments used in this work were loaned by the U. S. Weather Bureal thru the courtesy of the Division of Agricultural Meteorology and Instruments.

near this a twelve-inch tipping bucket rain gauge. These were connected with a two-magnet register placed in a shelter house at the endge of the field. Upon this shelter was a wind vane mounted with an improvised recording mechanism placed inside. During the first of June all of the seedlings were carefully examined for early appearance of the disease upon the cotyledons and first leaves. None was found at this time, but judging from later developments a few probably escaped notice. Upon each of the following dates (June 16; 29; July 5; 21) eight to twelve consecutive plants were inoculated with the bacteria in each of two places. One was upon the long rows (extending north-east to south west) and the other was on two short rows. A few days later all of the plants immediately surrounding these were numbered and examined for the disease. The plants were charted to scale, the numbers and amount of the disease recorded. Upon close examinattion af the Charts I, II, and III it is noted that in Experiments A and C definite directional spread of the disease took place. This direction is toward the south-east. There were isolated diseased plants in other places but the means of this spread has not been determined, probably though by insects. The simultaneous spread in experiments B and D are in agreement with that in Experiments A and C, being in these cases across rows to both sides, but in the same general direction.

Without any doubt there has been "directional" spread of the disease in these experiments. Further work on this part of the subject is under way at the present time with more satisfactory apparatus. The equipment now includes a standard six-foot wind vane with electric contacts and a quadruple register for recording wind direction, velocity and rainfall, together with a special anemoscope for the investigation of minute oscillations in wind direction during rainfall. (Plate III). Studies have been made also upon the duration and velocity of wind while rain falls in the cotton belt. The frequency with which relatively high velocities occur is of interest though not of great importance. In view of the fact that the bacteria causing this type of disease must become present in the leaf surface film, a certain amount of time after the beginning of rainfall must be allowed for this to take place. It is difficult to determine such a period accurately, because the process of emanation from the lesions begins immediately after the deposition of water upon the infected

part, very slowly at first but increasing rapidly with the increase of time. The rate at the beginning must be dependent upon the previous atmospheric humidity and temperature. A half hour has been arbitrarily determined upon as sufficient time to allow the surface film of any diseased leaf to contain sufficient bacteria to permit this means of spread to become effective.

That little spread takes place from old lesions has been clearly shown in these experiments. Others have noted the fact that the bacteria are isolated with great difficulty from spots which are old and have dried out. In Experiment IV, illustrated in Chart IV, plants inoculated upon July 5th were infected and showed characteristic lesions upon July 20th. In Experiments VI, illustrated by Chart V, plants inoculated July 20th were also infected in due time, but from there the spread was marked and in the direction of the wind. The subsequent records illustrated in both charts were made at the same times. The difference in spread can only be explained by the relative viability of the bacteria in the lesions at the time the proper meteorological conditions for spread prevailed. The lesions on the plants first inoculated developed and dried out, while those in Experiment VI were viable and virulent at the critical time.

The rains or storms of the Cotton Belt are of two classes: (1) the ordinary rain or thunderstorm of short duration; (2) the more severe storms of violent character and longer duration usually known as "hurricanes".* Representatives of each of these classes have been analyzed and tabulations made of velocities attained at seven stations during the period following the first half hour of rainfall. In order to accurately determine these facts the rain-hours duration of each period of precipitation has been calculated, each fifteen-minute period in which as much as one-hundredth of an inch of water fell being taken as the unit. The summation of the duration of velocities of certain rates prevaling during these periods has been made, units as low as five minutes being used. This is comparatively easily done after the data recorded upon the

*The data presented in this connection were secured from the original register sheets made at the U. S. Weather Bureau Stations in the different cities. The writer is again indebted to the Weather Bureau, particularly in this connection to the section of Climatology.

U. S. Weather Bureau register sheet (Form 1001) have been transposed to coordinate paper and the values plotted as synchronous curves.

TABLE XII.

WIND VELOCITY HOURS OF 209 REGISTRATIONS OF DISTINCT OR IDENTICAL STORMS AFTER THE FIRST HALF HOUR OF RAINFALL,

	Hours of total rainfall during the summer								Per
Miles	New Orl'ns	Vicksburg	Mobile	Birming'm	Macon	Columbia	Miama	Total	Cent
	48.05	46.	48.60	60.75	48.50	61.50	101.24	369.11	
5	34.46	30.73	48.51	47.49	30.40	47.98	84.65	324.22	88
10	19.46	12.90	31.01	21.91	9.39	19.73	42.99	157.39	43
15	10.96	6.65	16.81	10.66	2.73	7.32	14.74	69.87	19
20	8.66	2.82	7.48	4.75	.82	1.57	6.08	32.18	9
30	3.33	.16	.82	0.00	.24	.16	.25	4.96	1
40	0.00	0.00	.16	0.00	.08	0.00	0.00	.24	.06
50	0.00	0.00	0.00	0.00	0.00	0.0			
					0.00	0.00	0.00	0.00	0

TABLE XIII.

WIND VELOCITY HOURS OF 209 REGISTRATIONS OF DISTINCT OR IDENTICAL STORMS DURING THE FIRST HALF HOUR OF RAINFALL.

	Hours of total rainfall during the summer								Per
Miles	New Orl'ns	Vicksb'g	Mobile	Birm'ham	Macon	Columbia	Miama	Total	Cent.
	17	11	16.5	18.5	13.5	11.5	17.5	105.5	
5	14.79	9.72	16.14	13.43	10.75	10.31	15.60	90.94	91
10	8.21	6.06	12.33	8.60	5.34	6.90	8.90	56.34	58
15	4.06	3.15	8.48	4.29	1.76	3.81	2.74	28.29	28
20	1.81	1.40	4.07	2.29	.93	1.65	1.16	13.31	13
30	.66	.40	.91	.08	.02	.49	.08	2.64	3
40	0.00	.24	.16	0.00	0.00	.33	0.00	.73	0.7
50	0.00	.08	0.00	0.00	0.00	.08	0.00	.16	.15

TABLE XIV.

RAIN-HOURS OF HURRICANE OF SEPTEMBER 27-29, 1917.

	Pensacola		Mobile		Birmingham		Macon		Columbia	
Rain-hours	27.7		25.7		17.5		14.25		12.25	
Class	Hours	Percent	Hours	Percent	Hours	Percent	Hours	Percent	Hours	Percent
5	26.72	97	0		17.5	100	11.4	80	10.32	85
10	26.22	95	0		6.25	36	8.82	62	4.82	40
15	25.56	92	23.29	91			1.99	14	.41	3
20	23.48	85	20.73	81			.08	1		
30	20.32	73	16.57	64						
40	15.41	56	12.07	47						
50	13.66	49	8.66	34						
75	9.58	35	3.58	14						

It has been impossible to study and present the minute details of value contained in the data secured, but the more obvious and important facts are at hand. In Table VII are presented the rain-hours which occurred at each of the stations used in this work during the summer of 1917, (April 1 to October 1). As was stated above these have been determined by summation of fifteen-minute periods in which one-hundredth inch or more of water fell. For each station are given the rain-hours during which the wind exceeded the velocities listed in the column on the left. From the total rain-hours of all the stations the percentage of the rain-hours during which the wind exceeded the velocities listed at the left have been calculated. These percentages are indicative of the probabilities to be expected during rains in the cotton belt and represent the most valuable results obtained from the studies made. The probabilities of the various sections of this area, such as those immediately surrounding the stations investigated can not be determined because of the writer's inability to study the records for a period of years.

A concise statement of the result is as follows: during 1 percent of the time following the first half hour of rainfall the rain will probably exceed 30 miles per hour; during 9 per cent of the time, 20 miles; during 19 per cent, 15 miles; during 43 percent, 10 miles; and during 88 per cent, 5 miles per hour. In what proportion to the velocity, the seriousness of this method of disease dissemination may increase, can not be stated, yet the action exists in a still atmosphere and increases in a high ration with the increase in velocity.

As is to be expected, a difference in the wind velocity during rainfall has been found to exist between the coastal stations and the inland stations, and these data are presented in Table XV. The averages are indicative of the relative differences and, because of the greater area of the inland regions over the coastal in the cotton belt, should be given greater importance in the calculation of the percentage of probability of prevalence of the various conditions.

TABLE XV.

WIND VELOCITY DURING THE RAIN HOURS AFTER THE FIRST HALF HOUR OF RAINFALL, COASTAL AND ISLAND STATIONS COMPARED.

AVERAGE.

Miles	New Orl'ns	Mobile	Miama	Jackson-ville	Costal Average	Inland Average	Vicksburg	Birmingham	Macon	Columbia
		48.05	48.6	118.85	83.5		45	60.75	48.5	61.5
5	72	99	71		81	74	67	78	63	78
10	49	64	36	59	69	26	26	35	19	32
15	23	32	12	26	23	12	11	16	6	12
20	18	15	5	8	9	5	6	8	2	2
30	7	2	.2	2	3	.3	.3	0	.5	.3
40	0	.3	0	0	0	0	0	0	.2	0
50	0	0	0	0	0	0	0	0	0	0

CONTROL.

The separation of primary and secondary infections, together with the fact that but one source of primary infection has been found very obviously leads to an important point of attack for the development of a method for control. The partial success of sterilizing the seed coat and lint was noted by Rolfs (12), but in his experiments 3 per cent of the plants in one field and 7 per cent of the plants in another were diseased fifty-one days after they were planted. Whether Rolfs interpreted this as due to primary infections from bacteria which had escaped the treatment or were inside the seed is not known, but it is certain that ample time had elapsed to permit abundant secondary spread to have occurred from the untreated plants used as controls.

An attempt was made to repeat the experiment of Rolfs. Four small samples of seed were treated with concentrated sulphuric acid until the lint was removed and then washed in running water. This was followed by immersion in a solution of mercuric chloride (1 part in 1000 parts of water). It seems improbable that any living organisms should survive the action of the acid, but since there was very often a small tuft of fibers remaining at one end of the seed, this part of the treatment was added as a precautionary measure. The success of this treatment in preventing the primary spots upon the cotyledons of the seedlings is shown by the results noted in Table XVII. In these experiments, each lot of seed was planted in successive rows so that after the first appearance of the disease upon the plants from untreated seeds, it spreads to the neighboring rows. This spread, as has been

noted before, is very rapid. It is only when diseased plants are well isolated in such experiments that it can be hoped to save them from infection for a whole season. This has been attempted by separating plots on which treated seed is planted from those containing the checks by seven or eight rows of corn.

TABLE XVI.

Results of treatment of cotton seeds with sulphuric acid and mercuric chloride.

Experiment 6	Treated	Record of disease		
			28 days	0.5%
Lot 1.	May 14 10 days 0%	16 days 0%	28 days	0.5
Control	May 14 10 days. 4	16 days 2.6	28 days	38.
Lot 2	May 14 10 days 0	16 days 0	28 days	.1
Control	May 14 10 days 0	16 days 4.2	28 days	50.

The control demonstrations were conducted during the spring in four parts of the state; namely, Clemson College, Summerville, Florence Experimental Farms, and the Stono Plantation, James Island. At each place different lots of seed were used. At the Stono Plantation, Sea Island cotton growing on the place was treated and planted. In each experiment six half-acre plots were separated from each other by rows of corn and the treated and untreated seed were planted in alternate plots. Plate IX. In this way, yields in triplicate plots of diseased and healthy plants could be compared.

In the records of observation in Table XVII the percentage of plants affected by this disease are given on two dates. A plant was considered diseased if but one spot caused by *Bacterium Malvacearum* was found. While the treated and untreated plots are grouped for the purpose of tabulation of the results, they alternated in the field, and the single untreated plots serve as satisfactory controls on the results of the treatment. For the determination of an accurate percentage of diseased plants in the treatment plots five hundred to four thousand plants were used in each case, while in the case of the untreated plots smaller numbers were used for these estimations. It is to be noted that the control of the disease in the Clemson Experiment was not as satisfactory as in the others. The explanation for this is not at hand. One or more of several factors as well as a failure in the treatment, may have influenced this result. It is true that the disease was more abundant in the controls of the Clemson Experiment, and the Meteorological conditions during early summer were ideal for its prolific dissemination.

Results of the demonstrations of the control of the Angular Leaf Spot of Cotton at four places in triplicate.

TABLE XVII.

Treated Plots	Clemson College Exp.	Florence Exp.	Stono Plantation Exp.	Summerville Exp.
1	0—1.09	.4—.04	0—.15	.03
2	0—7.56	.1—.54	0—.2	.15
3	0—4.46	0.—.09	0—.71	0
Untreated Plots				
1	37.—99.	46.—47.	4.4—13	49.
2	22.—99.	52.—33.	3.7—23.	54.
3	27.—99.	56.—48.	10.6 —31	60

A numerical count of experiments at Summerville was not made during the seedling stage but a hasty examination showed that the disease was not present upon the plants from treated seed and was upon 25-30 percent of those from untreated seed.

At Florence shortly after the seedlings appeared, a rainy period prevailed during which seven inches of rain fell during several days. The corn at that place was practically a failure as a barrier between the plots of treated and untreated plants. Ample time had also elapsed and it is doubtless a fact that the disease on the five plants in the treated plots were secondary in origin, coming from neighboring treated (?) plots by some agent or other. As was said before, it is only when diseased plants are a considerable distance from control experiments that the danger of infection is avoided. This has been accomplished at two places on James Island, namely at the Stono plantation and Centerville plantations where twenty acres in one field and thirty acres in another contain plants free from treated Sea Island Cotton seed. Examinations have been made of these fields and the disease has not thus far been found upon plants in the center of them, where diseased plants are not near enough for spread. Even here occasional infection by insects, birds and laborers, particularly the latter, is to be expected, but conditions will never approximate these prevailing in the least diseased fields throughout the state.

The treatment of cotton seed with Sulphuric acid on a large scale for farm planting seemed too impracticable even to attempt. When cooperators wished to undertake the prob

lems, the writer was anxious to demonstrate the efficiency of the treatment under field conditions and succeeded in devising methods and apparatus which proved satisfactory. Six wooden tubs were secured. Quarter inch holes were bored very closely in the bottom of two of them making sieves in which the seed could be washed in running water. Two others of the tubs were coated with melted roofing pitch which proved in previous experiments to be the coating most resistant to the action of the acid available. One of these tubs was nearly filled with seed to be treated and a quantity of the acid poured over them from a carboy. The seed were then thoroughly stirred into the acid and as they became wet they were easily submerged. After the lint has been removed (in 8-10 minutes) seed and acid are poured into one of the perforated tubs supported over the second coated tub. The acid quickly passes thru and the sieve is then placed over a hole dug in the ground and the acid thoroughly washed out with running water. This is the most critical part of the operation for the temperature of the mass of seed becomes very great if sufficient water is not applied to keep them cool. A tub of water was kept with which the seed were just flooded and the acid diluted immediately. After thorough rinsing in this way with running water, the seed were then poured into a solution of mercuric chloride in one of the remaining tubs. The solution was made approximately, 1 part in 1000 parts of water by estimating the capacity of the tub and adding proportional sufficient amount of the salt. After ten minutes immersion in this solution the seed were poured into the second sieve prepared, which was supported on another tub. The solution was preserved for later use and the seed again washed in running water, after which they were emptied out upon a clean roof to dry. The acid can be used until it is too thick to pass thru the sieve, when it should be washed out with the rinsing water. This is the most expensive part of the process, but when purchased in bulk the cost can be greatly lessened. During the work described above, seventy bushels of seed were treated in ten hours by four men, using seventy gallons of sulphuric acid which cost $0.75 per gallon.

SUMMARY.

The disease caused by Bacterium Malvacearum is most noticeable upon the foliage leaves of cotton, but may also be found upon cotyledons, stalks, petioles, bracts and bolls. On

the leaves it is first manifested as dark green angular spots on the under surface. These later appear upon the upper surface and become reddish brown. The spots never cross the larger veins, hence their angular form.

The organism causing Angular Leaf Spot of cotton is a short rod-shaped bacterium which usually occurs in pairs or chains of three or more. Flagellae have been seen on organisms from young cultures.

The seasonal history is divided into two phases—(1) the primary infections upon the cotyledons and (2) the secondary infections upon the foliage, leaves, stems, bracts and bolls.

The factors most probably concerned in the hibernation and appearance of the disease are

(1) Presence of bacteria in the soil.
(2) Survival in decaying cotton plants and plant parts.
(3) Contamination of short lint or fuzz upon the seed.
(4) Internal seed infection.
(5) Hibernation upon or within insects.
(6) Susceptibility of plants closely related to the cotton plant.

The first two factors seem to be of little importance. The third plays an important part, as the bacteria spread over the lint of an entire boll and are also transferred in the process of ginning. The fourth factor, internal seed infection, seems to occur only in rare cases. Infection by bacteria carried over winter by insects probably does not occur. Nothing resembling Angular Leaf Spot has been found upon the wild Malvas.

The amount of injury caused by this disease has not been fully determined. The leaf loss is very low but the effect on the yield is not definitely known. Boll infection in the Piedmont section is slight, never having been found to exceed one percent. It does, however, form a means of entrance for several serious secondary infections. In the more southerly sections of the country boll infection has been found to run as high as sixty percent. Eighty percent of bacterial spots on seventy-five bolls examined had some secondary infection.

Wind during rain is an important agent of dissemination: both local and general. Little evidence of insect dissemination was found. Dissemination experiments show that the spread of the disease took place in the direction of the prevailing wind during a storm.

Sterilization of the seed coat has been found the most efficient means of control. This was accomplished by the use of sulphuric acid and mercury bichloride.

PLATE I.

The angular leaf spot of cotton. Depressed, discolored, papery spots bounded by smaller veinlets of leaf. (Mag. x2).

PLATE II.

"Zigzag" lesions formed by coalescence of smaller spots along larger veins. (Natural size).

PLATE III.—Instruments used during the summer of 1918 for recording meteorological conditions influencing the dissemination of the angular leaf spot of cotton. (Loaned by U. S. Weather Bureau).

PLATE IV. Demonstration of control of the angular leaf spot of cotton at the Coast Experiment Station, Summerville, S. C. Note four of six plots in view, each separated from the others by seven rows of corn. Treated and untreated plots alternate.

PLATE V.—Treatment of cotton seed. The seed are in a tub coated with roofing pitch. Sulphuric acid is being poured over the seed from a carboy.

PLATE VI. Treatment of cotton seed. Drying of seed after treatment with sulphuric acid and mercuric chloride.

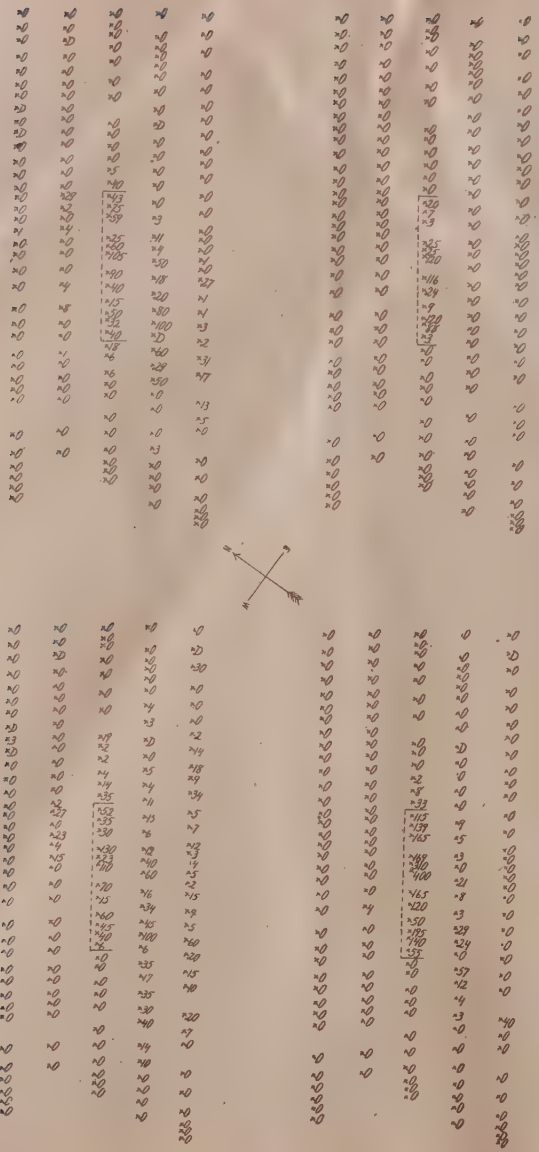

CHART I.—Graphic illustration of the spread of the angular leaf spot of cotton from a point of inoculation. Plants in the center row inoculated June 16, 1917. Upper left—spots upon inoculated plants July 5, no neighboring plants diseased. Upper right—disease July 20. Lower left—that present Aug. 5, lower right—disease present Aug. 25. Note spread in a south-easterly direction.

CHART II.—Illustration of spread of disease upon plants in rows running at right angles to those in Chart I. Left to right—inoculation and records on same dates as those in Chart I. Note spread in southerly and easterly directions.

CHART III.—Illustration of spread of this disease from a point of inoculation. Plants in center row inoculated July 5, 1917. Above—disease on inoculated plants only on July 20. Lower left —that present Aug. 5. Note spread toward south-east.

CHART IV.—Illustration of spread of disease upon plants in rows running at right angles to those in Chart III. Left to right—inoculation and records on same dates as those in Chart III. Note comparatively slight spread of disease except that toward the southeast.

CHART V.—Illustration of spread of disease upon plants in rows running as in Chart IV. Inoculated July 8; first record about Aug. 1; second and third same as in Chart IV. Note more extensive spread in latter case due to relative viability of bacteria at the time proper meteorological conditions for spread prevailed.

BIBLIOGRAPHY.

(1) Atkinson, Geo. F. Black rust of cotton. Alabama Agricultural Experiment Station, Bull. 27; 1-16. 1891.

(2) ———— Some diseases of cotton. Alabama Agricultural Experiment Station, Bull. 41; 1-65. 1892.

(3) Barre, H. W. Cotton shedding. South Carolina Experiment Station, Report 26, 17-18. 1913.

(4) Edgerton, C. W. The rots of the cotton boll. Louisiana Agricultural Experiment Station, Bull. 137; 8-19. 1912.

(5) Faulwetter, R. C. The physiology of *Bacterium malvacearum* Smith. South Carolina Agricultural Experiment Station, Report 29.

(6) ———— The dissemination of the angular leaf spot of cotton. Journal of Agricultural Research, 8; 457-475. 1917.

(7) ———— Wind blown rain, a factor in disease dissemination. Journal of Agricultural Research, 10, 639-648. 1917.

(8) ———— The Alternaria Leaf Spot of Cotton. Phytopathology. 1918.

(9) Lloyd, F. E. Leaf water and stomatal movement in Gossypium, etc. Bull. Torrey Botanical Club, 40; 1-27. 1913.

(10) Lohnis, F., and Smith, N. R. Life cycles of bacteria. Journal of Agricultural Research. 6; 675-702. Plates A-G. 1916.

(11) Orton, W. A. Sea Island cotton—its culture, improvement and diseases. U. S. D. A. Farmers' Bulletin, 302; 41-43. 1917.

(12) Rolfs, F. M. The angular leaf spot of cotton. South Carolina Agricultural Experiment Station. Bull. 184; 8-30, 1915.

(13) Smith, Erwin, F. The cultural characters of *Pseudomonas hyacinthii*. U. S. D. A. Div. Veg. Phys. and Path. Bull. 28; 153. 1901.

(14) ———— Bacterial in relation to plant diseases. Vol. 1. 95, 126, 165-166. 1905.